REMARKS

The claims under consideration are claims 1, 4-13, 23-24, 27-29, 68, 70, and 72-82. It is noted that claim 22 has been withdrawn, but was erroneously included in the Office Actions list of claims under rejection.

Amendments to the Specification

The Specification has been amended to incorporate the text of the figure legends into the BRIEF DESCRIPTION OF THE DRAWINGS. The spelling of "microsatellite" has been corrected in the description of Figure 2. No new matter is added.

Amendments to the Drawings

Applicants acknowledge the requirement for corrected drawings and hereby submit substitute sheets of corrected formal drawings for the Examiner's review and Office's approval. Applicants respectfully request entry of the substitute sheets, modified as described above.

Claim Amendments

Claim 1 has been amended to recite that the cell is a mammalian or plant cell. Support for this amendment may be found throughout the Specification, for example at page 14 lines 11-13, wherein it is stated that examples of hosts are cells or organisms including, among others, plants and mammals.

Claim 72 has been amended to specify that the organism is a plant. Support for this amendment may be found throughout the Specification, for example at page 14 lines

11-13, wherein it is stated that examples of hosts are organisms including, among others, plants.

Thus, no new matter is added by the amendments made herein.

35 U.S.C. §112, First Paragraph (Enablement)

The Office Action rejects claims 72-82 under 35 U.S.C. §112, first paragraph as allegedly non-enabled because the Applicants did not amend the claims to recite "A method for generating a genotoxic mutation in mismatch repair gene in a cell in vitro or in a cell of a non-human organism." As explained in the previous response, such a phrase would be *incorrect*. It is believed that the compounds of the invention ***inhibit the function*** of the mismatch repair genes, but the compounds ***do not cause mutations*** in the mismatch repair genes themselves as a mode of action. Thus, mutations that form in the DNA of the cells or organisms are due to the inability of the cells to correct the mutations that randomly occur during DNA replication (because the compound is inhibiting the proteins responsible for mismatch repair). As a result, mutations accumulate in the cells at an increased frequency.

Applicants invite the Examiner's attention to the specification at page 10, lines 6-8 wherein it is stated:

> As used herein "inhibitor of mismatch repair" refers to an agent that interferes with at least one function of the mismatch repair system of a cell and thereby renders the cell more susceptible to mutation.

The Specification further provides examples in which a cell or organism was exposed to a chemical inhibitor of mismatch repair, resulting in a hypermutable phenotype. Applicants direct the Examiner's attention to the Specification at Example 5,

wherein it is shown that human cells treated *in vitro* with dimethylanthracene became hypermutable as measured by microsatellite instability (a hallmark of mismatch repair deficiency). That is, the cells so treated had their mismatch repair system inhibited such that microsatellite regions developed mutations (either expansions or contractions Bat26 marker). As shown in Figures 4B and 5 and described in the Specification at page 27, lines 14-21, the wild-type cells were homozygous for a 26nt polyA repeat, whereas treated cells showed both contractions (24nt polyA) and expansions (28nt polyA).

The Specification also shows the effect of chemical inhibitors or mismatch repair in whole organisms. For example, *Arabidopsis thaliana* plants were treated with dimethylanthracene and examined for phenotype alterations (see Example 6). Treated plants were yellow and stunted as compared to wild-type plants (see Figure 6). The effect of the inhibition of mismatch repair and resulting hypermutability was confirmed by examining endogenous repeat markers (see Specification at page 29, lines 13-14) using the assay described.

Whole plants were also transfected with a reporter gene vector that expresses ß-glucoronidase in which a 20-base adenine repeat was placed upstream of the initiation codon resulting in an in-frame coding sequence (used as a control for glucoronidase ("GUS") activity). Another vector was generated in which a 19-base adenine repeat was placed upstream of the initiation codon resulting in an out-of-frame coding sequence. Some cells treated with the chemical inhibitor expressed glucoronidase due to microsatellite instability ("MI") with a resulting expansion of the polyA repeat, restoring the proper reading frame of the coding sequence. As stated in the Specification at page 34, lines 6-10:

> The presence of GUS activity in DMA treated plants indicates that the polyA repeat was altered, therefore, resulting in a frame restoring mutation. Agents such as EMS, which are known to damage DNA by alkylation cannot affect the stability of a polynucleotide repeat. This data indicates that plants are defective for MMR, the only process known to be responsible for MI.

In yet another Example, transformed yeast cells carrying a vector comprising a glucoronidase gene for use in a similar assay as that described for the plants. In this study (see Example 7), a subset of yeast treated with the chemical inhibitor of mismatch repair were able to produce glucoronidase due to the mutation at the microsatellite area. As stated in the Specification at page 35, lines 6-8,

> After incubation, a subset of yeast expressing GUS-OF contain blue subclones, while none are seen in EMS or control cells. These data demonstrate the ability of chemicals to block MMR of microbes *in vivo* to produce subclones with new output traits.

As the specification describes the use of the chemical inhibitors of mismatch repair to inhibit mismatch repair in cells *in vitro* and in whole organisms *in vivo*, including a wide range of species, Applicants assert that claims 72-82 are fully enabled by the Specification in their current form. Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Alleged Prior Art

Rejections under 35 U.S.C. §102(b)

The Office Action rejects the claims under 35 U.S.C. §102(b) as anticipated by Traczewska *et al.* (1991) *Acta Microbiologica Polonica* 40(3/4):235-241 ("Traczewska"), LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488 ("LaVoie") or Cerniglia *et al.* (1990) *Appl. Envron. Microbiol.* 56(3):661-668 ("Cerniglia"). Each will be addressed in turn.

1. Traczewska *et al.* (1991) *Acta Microbiologica Polonica* 40(3/4):235-241 ("Traczewska")

The Office Action rejects claims 1-13, 23-24, 27-29, 68, and 70 under 35 U.S.C. §102(b) as anticipated by Traczewska *et al.* (1991) *Acta Microbiologica Polonica* 40(3/4):235-241 ("Traczewska"). Claims 1 and 72 have been amended to recite that the cells are mammalian or plant cells or that the organisms are plants. Traczewska discloses exposing bacterial cells to anthracene. Thus, claims 1, 72 and the dependent claims are not anticipated by Traczewska. Claim 23 and the appurtenant dependent claims recite a method of making a mutation in a gene of interest and testing the cells to determine whether the gene of interest harbors a mutation. The second step is not taught by Traczewska. Thus, these claims are also not anticipated by Traczewska. Withdrawal of the rejection under 35 U.S.C. §102(b) is respectfully requested.

2. LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488 ("LaVoie")

The Office Action rejects claims 1, 4-13, 23-24, 27-29, 68, 70, and 72-82 under 35 U.S.C. §102(b) as anticipated by LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488 ("LaVoie"). Claims 1 and 72 have been amended to recite that the cells are mammalian or plant cells or that the organisms are plants. LaVoie discloses exposing bacterial cells to anthracene. Thus, claims 1, 72 and the dependent claims are not anticipated by LaVoie. Claim 23 and the appurtenant dependent claims recite a method of making a mutation in a gene of interest and testing the cells to determine whether the gene of

interest harbors a mutation. The second step is not taught by LaVoie. Thus, these claims are also not anticipated by LaVoie.

The Office Action further states that LaVoie teaches that methylated anthracenes have a tumor-initiating effect on mouse skin. Without conceding the correctness of the Examiner's argument, Claim 72 has been amended to recite that the organisms are plants. LaVoie does not teach mutagenesis of mammalian cells *in vitro*. Thus, the claims are not anticipated by LaVoie. Withdrawal of the rejection under 35 U.S.C. §102(b) is respectfully requested.

3. Cerniglia *et al.* (1990) *Appl. Envron. Microbiol.* 56(3):661-668 ("Cerniglia")

The Office Action rejects claims 1, 4-13, 23-24, 27-29, 68, 70, and 72-82 under 35 U.S.C. §102(b) as anticipated by Cerniglia *et al.* (1990) *Appl. Envron. Microbiol.* 56(3):661-668 ("Cerniglia"). Claims 1 and 72 have been amended to recite that the cells are mammalian or plant cells or that the organisms are plants. Cerniglia discloses exposing a type of fungal cell to anthracene. Thus, claims 1, 72 and the dependent claims are not anticipated by Cerniglia. Claim 23 and the appurtenant dependent claims recite a method of making a mutation in a gene of interest and testing the cells to determine whether the gene of interest harbors a mutation. The second step is not taught by Cerniglia. Thus, these claims are also not anticipated by Cerniglia. Withdrawal of the rejection under 35 U.S.C. §102(b) is respectfully requested.

Rejections under 35 U.S.C. §103(a)

The Office Action rejects claims 1, 4-13, 23-24, 27-29, 68, 70, and 72-82 under 35 U.S.C. §103(a) over any of LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488 ("LaVoie"), Chakravarti *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:10422-10426 ("Chakravarti"), or U.S. Patent Application No. 2002/0064879 A1 to Zhang ("Zhang") in view of Hoorn *et al.* (1993) *Mutagenesis* 8(1):7-10 ("Hoorn") and Myers *et al.* (1988) *Biochem. Biophys. Res. Comm.* 151(3):1441-1445 ("Myers"). Each will be addressed in turn.

1. LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488

The Office Action frankly admits that LaVoie does not teach that the anthracenes disclosed would cause hypermutation in any cell *in vitro* or *in vivo*. According to the Office Action, LaVoie teaches the application of various anthracenes to mouse skin and to cultures of *Salmonella typhimurium*.

Claim 1, 4-13, 23-24, and 27-29 are drawn to a method of making a hypermutable mammalian or plant cell *in vitro*

Claims 72-82, as amended, are drawn to hypermutable plant cells. Lavoie neither teaches nor suggests that the compounds act as mutagens in plant cells. As Plantae are a separate kingdom than Bacteria (such as *S. typhimurium*) and Animalia (such as mice), and plant cells have a cell wall which is not present in either *S. typhimurium* or mouse cells, it would not be obvious that the compounds described in LaVoie would have any effect in plants. Thus, there is an inadequate motivation to use the compounds of LaVoie in plants, and there is no reasonable expectation of success that such compounds would have any effect. Thus, claims 72-82 are not obvious over LaVoie.

2. LaVoie *et al.* (1985) *Carcinogenesis* 6:1483-1488 in view of Hoorn *et al.* (1993) *Mutagenesis* 8(1):7-10 and Myers *et al.* (1988) *Biochem. Biophys. Res. Comm.* 151(3):1441-1445

LaVoie in view of Hoorn *et al.* (1993) *Mutagenesis* 8(1):7-10 (hereinafter, "Hoorn") and Myers *et al.* (1988) *Biochem. Biophys. Res. Comm.* 151(3):1441-1445 (hereinafter, "Myers") does not achieve the Applicants' invention.

LaVoie describes, *inter alia*, the exposure of bacteria to dimethylanthracene. LaVoie also discusses that dimethylanthracene is weakly tumorigenic on mouse skin. Critically, however, LaVoie teaches on page 1487, column 2, that recent studies have suggested that 9,10-dimethylanthracene did not have significant tumor initiating activity. LaVoie attributes this to the lower dosage used and single administration to mouse skin. LaVoie also discusses that the metabolites of anthracene, 9-methylanthracene and 9,10-dimethylanthracene does not indicate quantitative differences that accounts for the "lack of mutagenic potency" (see LaVoie, page 1487, Col. 2, first full paragraph). Thus, one of skill in the art would not look to the teachings of LaVoie for the use of anthracene or 9,10-dimethylanthracene for use as a method for inhibiting mismatch repair in cells *in vitro* or in plants. The teachings of LaVoie teach away from their use in favor of other compounds.

The combination of LaVoie with Hoorn and Myers adds nothing. First, Hoorn does not teach or suggest use of the Applicants' claimed compounds for use. Rather Hoorn teaches the use of ethylnitrosourea (ENU), chlorambucil, cyclophosphamide, acrylamide, and 9,10-dimethyl**benz[a]**anthracene (DMBA) (see Hoorn at page 7,

Materials And Methods, "Test materials and Reagents"), which do not fall within the scope of the claims. Hoorn used the compound DMBA which LaVoie teaches as a much stronger mutagen than 9,10-dimethylanthracene. One of skill in the art would not be motivated to combine the teachings to use of a weaker compound.

Myers adds nothing as Myers teaches at page 1444, lines 3-6, that anthracene "is generally considered to be carcinogenically inert," and that 9,10-dimethylanthracene is "weakly active" in mouse skin. Thus, even in the hypothetical combination suggested by the Office Action, one of skill in the art would not be motivated to combine the teachings of LaVoie, Hoorn and Myers, rather one would be taught to employ stronger mutagens in an effort to induce genetic changes.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103 over LaVoie in view of Hoorn and Myers.

3. Chakravarti *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:10422-10426 in view of Hoorn *et al.* (1993) *Mutagenesis* 8(1):7-10 and Myers *et al.* (1988) *Biochem. Biophys. Res. Comm.* 151(3):1441-1445

Chakravarti *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:10422-10426 ("Chakravarti") teaches that DMBA treatment of mouse skin has a carcinogenic effect. Notably, no anthracene derivatives of the Applicant's invention are taught by this reference as DMBA does not fall within the scope of the claims (note Applicants response of November 15, 2002 at page 7 lines 1-9). Moreover, Chakravarti teaches at page 10426, final two paragraphs, that treatment of mouse skin with DMBA resulted in apurinic sites of about 40,000 sites per cell in the 4 hour treatment period, as opposed to

the reported 10,000 sites per cell per day of untreated cells (restated as about 2000 sites spontaneously formed in a 4 hour period, according to Chakravarti). Chakravarti concludes that the rate is at least 10 fold more apurinic sites with treatment. Chakravarti further speculates in the last paragraph that excessive apurinic sites may overwhelm the repair capacity of the cells, or that the stable adducts could interfere with the repair process. Thus, one of skill in the art would be motivated to use a strong mutagen to interfere with this process and create as many apurinic sites as possible.

Hoorn simply does not teach or suggest the use of any compounds of the invention. Rather Hoorn teaches the use of ethylnitrosourea (ENU), chlorambucil, cyclophosphamide, acrylamide, and 9,10-dimethyl**benz[a]**anthracene (DMBA) (see Hoorn at page 7, Materials And Methods, "Test materials and Reagents"), which do not fall within the scope of the claims.

Myers teaches at page 1444, lines 3-6, that anthracene "is generally considered to be carcinogenically inert," and that 9,10-dimethylanthracene is "weakly active" in mouse skin. As Chakravarti speculates that the use of strong mutagens may overwhelm the repair process, one of skill in the art would not be motivated to combine the teachings of Myers (which teaches a weak mutagen, at best) with Chakravarti with a reasonable expectation that a weak mutagen would overwhelm the repair process.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103 over Chakravarti in view of Hoorn and Myers.

4. U.S. Patent Application No. 2002/0064879 A1 to Zhang in view of Hoorn *et al.* (1993) *Mutagenesis* 8(1):7-10 and Myers *et al.* (1988) *Biochem. Biophys. Res. Comm.* 151(3):1441-1445

U.S. Patent Application No. 2002/0064879 A1 to Zhang ("Zhang") teaches a method for identifying the function of a gene sequence of a plant by comparing amplified portions of genes from wild-type plants with mutant plants. Zhang teaches that plants can be mutated using mutagenic substances such as diepoxybutane, diethyl sulfate, ethylene imine, ethyl methanesulfonate, and N-nitroso-N-ethylurea, or with ionizing radiation. Notably, Zhang does not teach or suggest the use of anthracenes that block mismatch repair. The mutagenic substances contemplated by Zhang include compounds that cause gross genetic abnormalities, not subtle mutations through the blocking of mismatch repair. It was a goal of Zhang to cause a significant enough change to be detected by PCR or other standard methods. According to Zhang, typically a change of greater than at least 30% of a distance between the primers that hybridize to the two known polynucleotides is preferred (see Zhang, paragraph [0066]. Note also that the in the method of Zhang, the genes to be analyzed must be known. The Applicants teach a different strategy in mutagenesis. That is, the use of inhibitors of the mismatch repair process to allow for subtle mutations in the genome, rather than gross abnormalities as contemplated by Zhang. Applicants invite the Examiner's attention to the Specification at page 18, lines 4-12 wherein it is described that mutagens have a wholly different effect on cells than the inhibitors of the invention.

Moreover, Hoorn does not teach or suggest the inhibitors of the invention. Rather Hoorn teaches the use of ethylnitrosourea (ENU), chlorambucil, cyclophosphamide,

acrylamide, and 9,10-dimethyl**benz[a]**anthracene (DMBA) (see Hoorn at page 7, Materials And Methods, "Test materials and Reagents"), which do not fall within the scope of the claims (See Applicants Response of November 15, 2002, page 7, lines 1-9 (referring to DMBA)). The combination of Hoorn with Zhang does not achieve the Applicants invention.

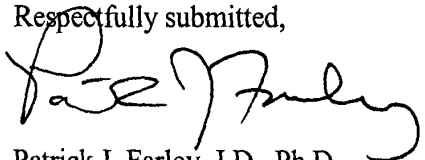
The teachings of Myers add nothing to the combination. Myers teaches at page 1444, lines 3-6, that anthracene "is generally considered to be carcinogenically inert," and that 9,10-dimethylanthracene is "weakly active" in mouse skin. As Zhang simply did not contemplate the subtle nature of mutagenesis and specifically teaches away from the use of a weakly carcinogenic substance. Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Hoorn and Myers with the method of Zhang with a reasonable expectation of success as Zhang teaches the use of strong mutagenic substances.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103 over Zhang in view of Hoorn and Myers.

CONCLUSION

Applicants submit that the claims are in condition for allowance and are neither anticipated nor obvious in view of the cited art. Applicants respectfully request allowance of the claims as amended.

Respectfully submitted,



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